Extended Egg Retention and Its Influence on Embryonic Development and Egg Water Balance: Implications for the Evolution of Viviparity

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Abstract

Viviparity in squamate reptiles presumably evolves via gradual increases in the time that eggs are retained within the oviducts. We evaluated the extent and consequences of phenotypic plasticity in egg retention for the oviparous lizard Sceloporus scalaris. *This species not only exhibits facultative egg retention, but has close relatives that are viviparous. Thus,* S. scalaris *may possess reproductive features that are transitional between oviparity and viviparity. We tested the hypothesis that oviposition normally occurs when further retention would impair embryonic development. To do so, we determined the effects of extended egg retention on embryonic development and on egg water balance for a population in which females normally retain eggs to Dufaure and Hubert stages 31.0–33.5. Under substrate conditions that inhibited oviposition, females retained eggs to stage 39.5, or 0.5 stage units short of hatching. Extending egg retention did not retard the development of embryos relative to that of embryos in control eggs. Water did not accumulate in the extraembryonic compartments of retained eggs as it did in control eggs; all water uptake was associated with the embryo. The pattern of embryonic development within retained eggs does not support the hypotheses (1) that oviposition occurs when gas exchange in utero is no longer sufficient to support the needs of the embryos, or (2) that increases in the duration of egg retention and decreases in eggshell thickness evolve concurrently. Our observations on water uptake additionally suggest that viviparity may evolve more easily in taxa that are able to preclude storage of excess water in the extraembryonic compartment of the egg while allowing retained embryos access to sufficient water for normal embryonic development.*

Introduction

The evolutionary transition from oviparity to viviparity in squamate reptiles is presumably a gradual process in which an increasingly greater proportion

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of embryonic development is completed within the female (Packard et al. 1977; Shine 1985; Guillette 1993). However, few species exhibit the presumptive intermediate stages of egg retention between oviparity and viviparity (Blackburn 1995). The majority of oviparous squamates retain eggs to embryonic stages 25–31, and the modal stage at oviposition is stage 30 (Shine [1983]; DeMarco [1993*a*]; the staging sequence used is that of Dufaure and Hubert [1961], where hatching occurs at stage 40). Consequently, most squamates retain eggs for only about one-fourth of their total developmental time (DeMarco 1993*a*).

Uniformity in embryonic stage at oviposition suggests a common physiological constraint (or constraints) that inhibits the extension of egg retention. Some lizard species lack the physiological capacity to facultatively extend the duration of egg retention. For example, oviposition by *Sceloporus undulatus* in the laboratory cannot be delayed very long; when embryos reach stages 30–31, the approximate stages at oviposition in the field (Gelbach 1965; Sexton and Marion 1974), females oviposit even if the substrate is unsuitable for successful incubation (T. Mathies, unpublished data). Other species are able to facultatively retain eggs beyond the normal time of oviposition, but embryonic development is retarded. For example, *Sceloporus virgatus,* a close relative of *S. undulatus,* can support embryonic development in utero to at least stage 36 in the laboratory, but embryos develop slowly and hatching is disproportionately delayed (Andrews and Rose 1994).

These observations suggest that oviposition occurs when gas exchange in utero is no longer sufficient to support the increasing needs of the embryos (Guillette et al. 1980; Shine 1983; Guillette 1993). This argument is supported indirectly by the rapid increases in the size and metabolic demands of embryos at later stages in development (Dmi'el 1970; Prange and Ackerman 1974; Guillette 1982; DeMarco 1993*b*) and by the reduction in thickness or the absence of the eggshell in viviparous species. Thus, successful retention of embryos beyond the normal range of stages at oviposition presumably requires a reduction in eggshell thickness as well as other modifications such as increased vascularity of the oviduct and hormonal support for egg retention (Guillette 1993). These modifications are part of a suite of characters that presumably evolve concurrently with increases in the duration of egg retention.

Water balance may place equally important constraints on retention of eggs beyond the normal stage at oviposition. The eggs of squamates are relatively "dry" when they are laid; to meet the requirements of the embryo, oviposited eggs must take up water throughout the remainder of developmental period, and eggs often take up water far in excess of the immediate needs of the embryo (Packard et al. 1985; Vleck 1991). Thus, water uptake

by eggs retained beyond the normal stage at oviposition could increase the costs of reproduction for the female in two ways. The first is by reducing the mobility of females because of the increased mass or bulk of the eggs (Shine 1980; Sinervo et al. 1991). The second is by increasing the size of the eggs and thus making oviposition more difficult or even impossible (Sinervo and Licht 1991). These considerations suggest that regulation of the egg water balance might play an important role in the evolution of extended egg retention.

Understanding the constraints on egg retention is thus an important component in determining mechanistic processes in the evolution of viviparity. We therefore tested the hypothesis that oviposition of squamate eggs occurs at the time when further retention would impair embryonic development because gas exchange, water availability, or both become limiting relative to conditions in the nest following oviposition. This hypothesis recognizes that short-term egg retention, at the expense of impaired embryonic development, is an important component of the reproductive strategy of species such as *S. virgatus* that retain eggs until summer rains provide the moisture necessary for successful incubation (Andrews and Rose 1994).

We tested this hypothesis by determining the effects of extended egg retention on embryonic development and on water balance of eggs using the oviparous lizard *Sceloporus scalaris*. This species is a particularly appropriate subject for investigating evolutionary questions concerning squamate viviparity. First, it is one of the few species known to exhibit stages of egg retention intermediate between oviparity and viviparity (Blackburn 1995). Females from high- and low-elevation populations oviposit at stages 35.5–37.0 and 31.0–33.5, respectively (Mathies and Andrews 1995). Second, it is a member of a species group containing both oviparous and viviparous members (Sites et al. 1992), and the close relationships of these taxa suggest that *S. scalaris* might possess relatively few reproductive features that constrain the evolution of viviparity. More important, reproductive features that facilitate successful egg retention beyond the normal embryonic stages at oviposition, if present, would presumably also be features associated with the evolution of viviparity.

Material and Methods

Collection and Maintenance of Females

Gravid female *Sceloporus scalaris* (low-elevation population) were collected at the Appleton-Whittell Research Ranch Sanctuary (1,460 m, $n = 30$) on the Sonoita Plain, Arizona, in late June and early July 1993. Females were

housed temporarily in terraria at the Southwestern Research Station at Portal, Arizona. These females were transported to Blacksburg, Virginia (625 m), on July 3 and placed in controlled environment cabinets (see below) on the following day.

Females were housed in tubs (60 × 38 × 22 cm) containing dry sand and clumps of bunch grass. We housed females on dry sand to inhibit oviposition, as females exhibit facultative egg retention in response to unsuitable conditions for oviposition (Mathies and Andrews 1995). To ensure that the hydration state of retained eggs was not influenced by inadequate water availability to the females, drinking water was provided by sprinkling the grass with water once or twice a day. In the field, females typically obtain water after rainfall by licking water droplets from grass stems (T. Mathies, personal observation). Females were fed crickets, wax moth larvae, and mealworms daily.

Experimental Design: Control versus Experimental (Retained) Clutches

Our objective was to compare the development of embryos and the water balance of eggs obtained at the normal time of oviposition (see next paragraph) and incubated in "nests" (control clutches) with those of eggs incubated in utero (experimental clutches). Gravid females were randomly assigned to control ($n = 14$) and experimental ($n = 16$) groups. Females assigned to the control group were removed from the controlled environment cabinets on July 6 and 7. Their clutches were removed surgically on these dates, and the clutches were returned to the controlled environment cabinets. Embryonic stages of these control clutches ranged from 27.0 to 34.0.

Oviposition at our field site in 1992 commenced on July 7 in association with the first heavy summer rains (Mathies and Andrews 1995), and embryonic stages of five freshly oviposited clutches collected from field enclosures from July 7 to 13 ranged from 31.0 to 33.5 (Mathies and Andrews 1995). In 1993, when this study was conducted, the first heavy rain occurred on July 8. We assumed that oviposition in 1993 occurred at this time. Thus, control clutches were obtained at the normal time of oviposition and at appropriate stages of embryonic development.

Control clutches were placed in plastic boxes with loose-fitting lids and incubated in a mixture of vermiculite and water (1.0 g dry vermiculite to 0.8 g distilled water, resulting in a water potential of −100 to −50 kPa [Andrews and Rose 1994]). This mixture was replaced once per week.

Incubation of Eggs and Females

Control clutches and experimental females were incubated under the same thermal conditions in three controlled temperature and light cabinets (Percival model I-30BL with B1 option) at 13.5L:10.5D and 18°–33°C (mean = 25.9°C). This regime approximated the light and body temperatures experienced by reproductive females and by eggs in nests in mid-July (Mathies and Andrews 1995). Eggs of *S. scalaris* incubated under the same conditions in another study had high (80%) hatching success (R. M. Andrews, unpublished data). Boxes containing eggs and tubs containing females were rotated between cabinets and among cabinet shelves every 3 d to control for location effects.

Sampling Protocols

Control and experimental clutches were selected randomly from their groups throughout the period July 12 through August 7 and sampled only once. Experimental clutches were obtained surgically. Within groups, clutches were sampled at least every 4 d. Eggs were weighed to 0.1 mg. The eggshells were removed from approximately half the eggs from each clutch, and the wet and dry masses of the entire egg contents determined. Embryos from the remaining eggs in each clutch were dissected free of their extraembryonic membranes, and their wet and dry masses were determined. Water content of the extraembryonic fraction of eggs was calculated by subtracting the mean embryo water content from the mean water content of entire eggs exclusive of the shell for each clutch. We assigned developmental stages to embryos using the staging sequence of Dufaure and Hubert (1961) with the following modification: if embryos were more advanced than a designated stage, but not as advanced as the next stage, then embryos were considered to be halfway, or 0.5 stage units, between the two stages. Only one embryo per clutch was staged because embryonic stage at oviposition does not vary within clutches (DeMarco 1992).

As part of another study, we measured the oxygen consumption of the control and experimental clutches (data not reported here) immediately after they were removed from females and just prior to sampling. We include data here on the water balance of eggs during the time oxygen consumption was measured because water fluxes in eggs during this period provide insight into the regulation (i.e., maternal vs. embryonic control) of the water content of intraoviductal eggs. In brief, we measured the oxygen consumption of each control and experimental clutch within small metabolic chambers for a 2–4-h period (30° ± 0.5°C, eggs resting on cheesecloth slightly moistened

with 0.9% NaCl irrigation, U.S.P.). Each egg was weighed at the beginning and at the end of the measurement period, and the change in the water content of the egg was determined as final egg mass minus initial egg mass. Calculations of the water content of the embryo and the extraembryonic fraction of the egg (above) assumed that only the water content of the extraembryonic fraction of the egg changed while eggs were in the metabolic chambers.

Statistical Analyses

All analyses were based on clutch means for each sampling unit. For example, if the dry masses of five embryos from one clutch were measured, then the dry embryo mass for that clutch was the mean for those five embryos. Statistical differences among regression lines were determined by ANCOVA following a test for homogeneity of slopes. The covariate was the number of days beyond July 8, the date when eggs were obtained in the laboratory. Means are reported as mean ± 1 SE. A significance criteria of $P < 0.05$ was used in all analyses. All statistical analyses were conducted with SAS software (SAS Institute 1985).

Results

Capacity of Females to Retain Eggs

None of the experimental females oviposited during the observation period. Extended egg retention (up to 30 d) did not appear to affect the health of the experimental females; 14 of the 16 experimental females remained in good health (two died during surgery) for at least 1 mo after their clutch was obtained.

Embryonic Development: Embryonic Stage, Dry Masses of Embryos, and Egg Contents

All embryos that we examined were alive and showed no obvious morphological abnormalities. Developmental stage of embryos increased linearly over time in both groups (Fig. 1*A*). A linear rather than an exponential relation between embryonic stage and time reflects the relatively narrow range of stages (32.0–39.5) observed. Embryonic stage did not differ between groups ($F_{1,27} = 0.02$, $P > 0.05$, ANCOVA). At the grand covariate mean (16 d), the adjusted mean embryo stages were 36.5 ± 0.3 and 36.4 ± 0.3 for the control and experimental groups, respectively.

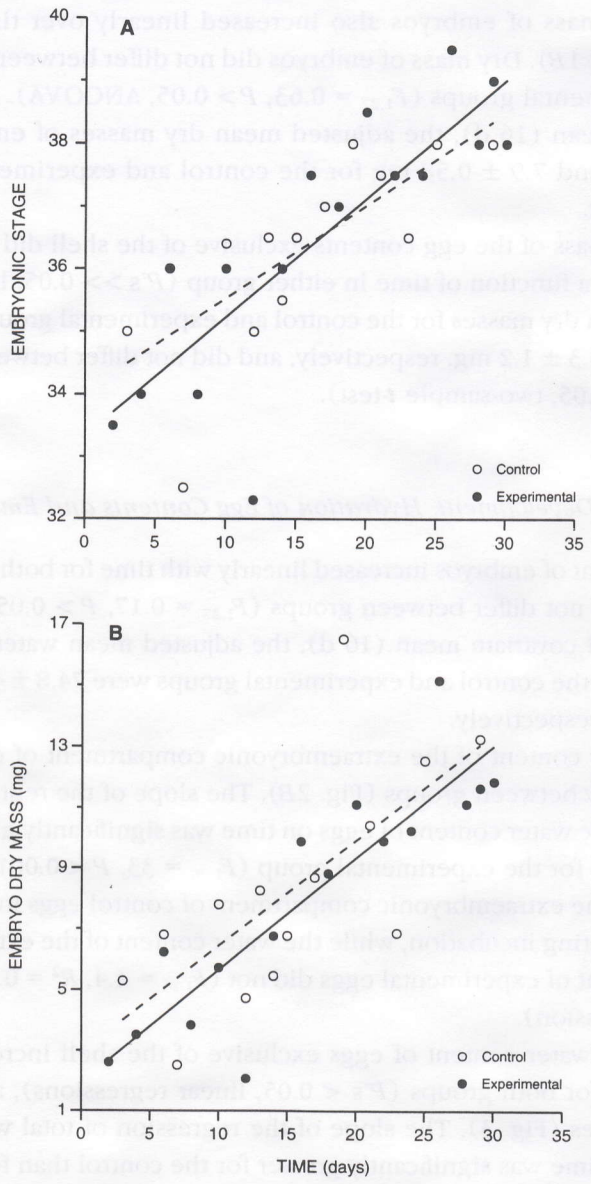

Fig. 1. Developmental stage (A) *and dry mass* (B) *of* Sceloporus scalaris *embryos in control (open circles, dashed regression line) and experimental (solid circles, solid regression line) groups as a function of days beyond time of normal oviposition. Regression slopes and intercepts did not differ significantly in either data set (see text).*

The dry mass of embryos also increased linearly over time for both groups (Fig. 1*B*). Dry mass of embryos did not differ between the control and experimental groups ($F_{1,27} = 0.63$, $P > 0.05$, ANCOVA). At the grand covariate mean (16 d), the adjusted mean dry masses of embryos were 8.6 ± 0.62 and 7.9 ± 0.58 mg for the control and experimental groups, respectively.

The dry mass of the egg contents exclusive of the shell did not vary significantly as a function of time in either group (P's >> 0.05, linear regressions). Mean dry masses for the control and experimental groups were 62.5 ± 1.7 and 63.3 ± 1.2 mg, respectively, and did not differ between groups ($t = 0.4$, $P > 0.05$, two-sample t-test).

Embryonic Development: Hydration of Egg Contents and Embryos

Water content of embryos increased linearly with time for both groups (Fig. 2*A*) and did not differ between groups ($F_{1,27} = 0.17$, $P > 0.05$, ANCOVA). At the grand covariate mean (16 d), the adjusted mean water contents of embryos for the control and experimental groups were 74.8 ± 4.48 and 72.3 ± 4.20 mg, respectively.

The water content of the extraembryonic compartment of eggs differed considerably between groups (Fig. 2*B*). The slope of the regression of extraembryonic water content of eggs on time was significantly greater for the control than for the experimental group ($F_{1,26} = 33$, $P < 0.001$). The water content of the extraembryonic compartment of control eggs increased considerably during incubation, while the water content of the extraembryonic compartment of experimental eggs did not ($F_{1,14} = 4.4$, $R^2 = 0.24$, $P > 0.05$, linear regression).

The total water content of eggs exclusive of the shell increased during incubation for both groups (P's < 0.05, linear regressions), albeit at very different rates (Fig. 3). The slope of the regression of total water content of eggs on time was significantly greater for the control than for the experimental group ($F_{1,26} = 28$, $P < 0.001$). Because the extraembryonic water content of experimental eggs did not vary during incubation, the increase in total water content of experimental eggs was entirely due to an increase in the water content of embryos.

The flux in total water content of eggs for the period that eggs were in metabolic chambers differed between groups. For the control group, 13 of 14 clutches decreased in mass, whereas for the experimental group, 15 of 16 clutches increased in mass ($\chi^2 = 22.5$, $P < 0.001$, chi-square test).

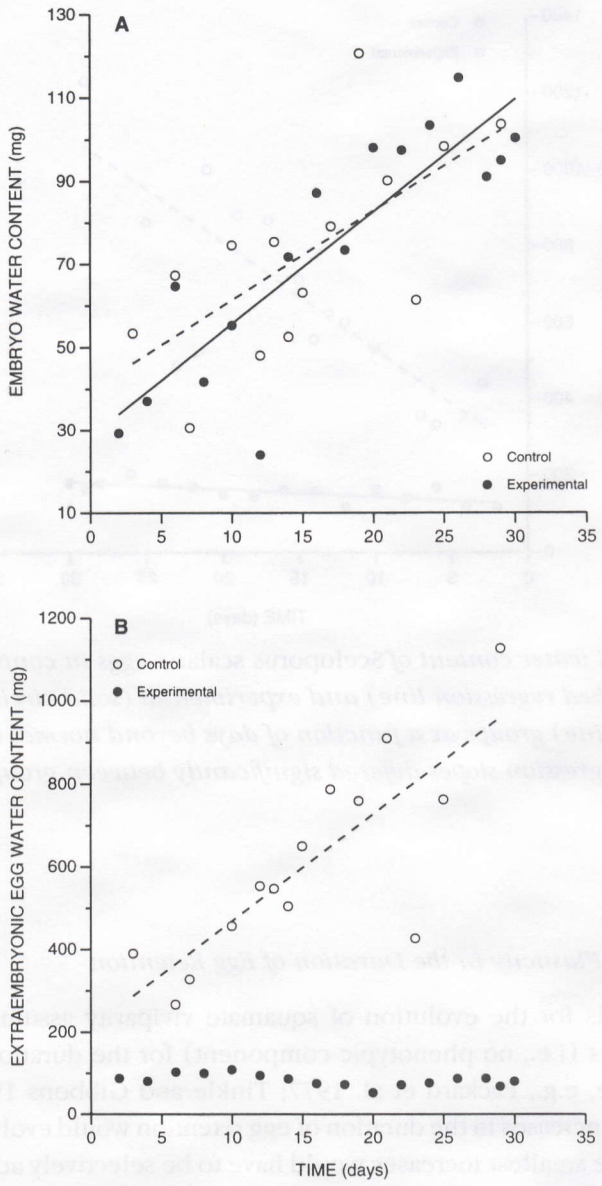

Fig. 2. Embryo water content (A) *and extraembryonic water content* (B) *of* Sceloporus scalaris *eggs in control (open circles, dashed regression line) and experimental (solid circles, solid regression line) groups as a function of days beyond time of normal oviposition. In* A, *regression slopes and intercepts did not differ significantly; in* B, *the regression for the experimental group was not significant (see text).*

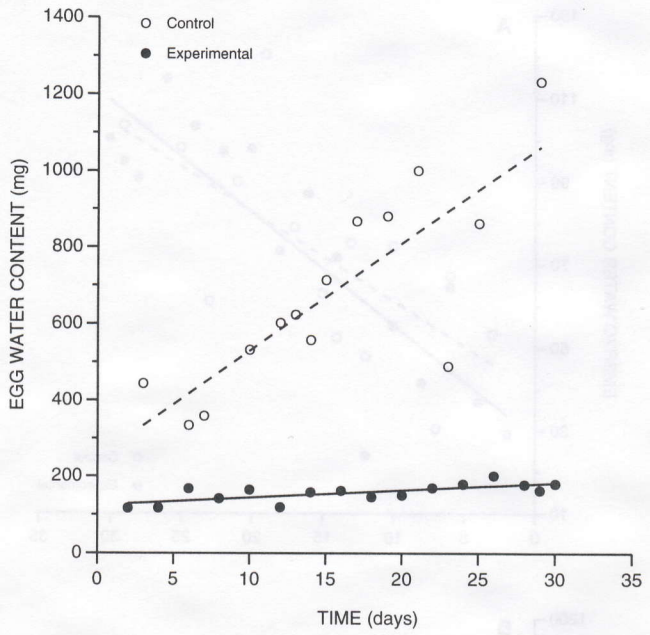

Fig. 3. Total water content of Sceloporus scalaris *eggs in control (open circles, dashed regression line) and experimental (solid circles, solid regression line) groups as a function of days beyond normal time of oviposition. Regression slopes differed significantly between groups (see text).*

Discussion

Phenotypic Plasticity in the Duration of Egg Retention

Early models for the evolution of squamate viviparity assumed a strictly genetic basis (i.e., no phenotypic component) for the duration of egg retention (see, e.g., Packard et al. 1977; Tinkle and Gibbons 1977). Under this model, increases in the duration of egg retention would evolve gradually, and even the smallest increases would have to be selectively advantageous. More recently, Shine and Guillette (1988) hypothesized that viviparity would be more likely to evolve in populations where at least some individuals exhibit phenotypic plasticity in the ability to retain eggs in response to an environmental stress. Such a mechanism could facilitate the evolution of viviparity by (1) enabling a relatively rapid shift in the mean duration of egg retention within a population and (2) conferring an increase in egg retention large enough to be selectively advantageous.

Oviposition by *Sceloporus scalaris* at our study site normally occurs when embryos are at stages 31.0–33.5. However, females can facultatively retain

eggs longer; in this study, females retained eggs as long as 20–30 d beyond July 8, the presumed date of oviposition in the field. We did not determine whether our experimental females could have laid eggs successfully. However, three *S. scalaris* females that were maintained in field enclosures in 1992, and had retained eggs 31 d beyond the normal time of oviposition, oviposited complete clutches when a suitable nesting substrate was provided, and eggs from these clutches all produced viable hatchlings (T. Mathies, unpublished data). Thus, we conclude that during periods of environmental stress (e.g., drought, cold temperatures), females can facultatively extend the duration of egg retention and oviposit when conditions become favorable. The ability of *S. scalaris* to substantially extend the duration of egg retention, the apparent commonality of this trait among individuals in the study population, and its membership within a species group containing viviparous members are features that are consistent with the hypothesis that phenotypic plasticity in the ability to retain eggs is a precursor to viviparity.

Embryonic Development: Control versus Experimental (Retained) Clutches

The apparent scarcity of oviparous taxa known to retain embryos much beyond stage 30 (Blackburn 1995) suggests that a common factor limits further egg retention. Perhaps the most widely held explanation for this observation is that embryonic development becomes limited by inadequate exchange of gases in utero beyond stage 30 (Packard et al. 1977; Guillette 1982; Shine 1983; Guillette 1993). Our data for *S. scalaris* do not support this idea. Even though females in our study population normally oviposit when embryos are at stages 31.0–33.5, embryonic differentiation and growth do not differ between control and experimental groups up to stage 39.5, just 0.5 stage units short of hatching (Fig. 1*A* and *B*). At this stage, embryos have reached approximately half of their mass at hatching. Regardless of the considerable embryonic growth remaining between stages 39.5 and 40.0, these data demonstrate that the timing of normal oviposition by *S. scalaris* is not associated with unfavorable conditions within the oviducts.

Does *S. scalaris* possess reproductive features that would explain how females are able to support normal embryonic development in utero long beyond the time when eggs are normally laid? The critical modification for maintaining adequate rates of gas exchange between the embryo and the female is presumed to be a reduction in the thickness of the eggshell (Packard et al. 1977; Shine and Guillette 1988; Shine 1991). Eggshells of *S. scalaris* from the study population are relatively thin (mean = 27 μm; Mathies and Andrews 1995) compared to those of other species of oviparous lizards that

also lay relatively small eggs (range = 48–79 μm; Cuellar 1979; Andrews and Sexton 1981; Trauth and Fagerberg 1984; Schleich and Kästle 1988).

Embryonic development within experimental females may have been facilitated by a relatively thin eggshell. Nonetheless, the comparison of eggshell thickness for high- and low-elevation populations of *S. scalaris* suggests that oviposition in this species, at least, is not functionally related to eggshell thickness; that is, the duration of egg retention and eggshell thickness do not necessarily evolve concurrently. Females from a high-elevation population of *S. scalaris* have relatively thin shells (mean = 19 μm) and oviposit when embryos are at stages 35.5–37.0 (Mathies and Andrews 1995). However, females from the low-elevation population are able to retain eggs to stage 39.5 without impairing embryonic development despite their relatively thick eggshell.

Hydration of Egg Contents and Embryos: Control versus Experimental Clutches

Flexible-shelled eggs of small lizards increase dramatically in size and mass as a result of water uptake after oviposition (Packard et al. 1985; Vleck 1991). Not all of the water taken from the environment is necessary for embryonic development, although excess water accumulation may provide a buffer against potential desiccation (Badham 1971). The water content of control eggs (exclusive of the embryo) of *S. scalaris* increased threefold during our 30-d observation period (Fig. 2*B*). In contrast, the water content of experimental eggs did not increase during development; all water uptake by eggs was due to increases in the water content of embryos. Thus, experimental eggs took up no more water than was commensurate with the developmental stage of the embryos.

Water uptake by experimental eggs was apparently limited by conditions in the oviduct. Experimental eggs immediately took up water after they were removed from oviducts and placed on the moist substrate inside metabolic chambers, while control eggs lost water under the same conditions. Water uptake by experimental eggs could have been limited by differences in solute potential between the oviduct and egg interior, by the pressure potential of the oviduct or abdominal cavity, or both. Eggs from clutches that were experimentally reduced in size and retained in utero took up more water than eggs from entire clutches that were retained in utero (T. Mathies, unpublished data). This observation suggests that pressure potential may have contributed to the low water uptake by experimental eggs while they were in utero. Regardless of the mechanism involved, the ability to inhibit excess water storage by eggs is advantageous when egg retention

is extended. For example, eggs of the small oviparous iguanid *Uta stansburiana* that have been experimentally enlarged often rupture during oviposition (Sinervo and Licht 1991). Moreover, retention past the normal stage at oviposition by the lizard *Cnemidophorus uniparens* resulted in death of females when eggs continued to absorb water and grew larger in the oviducts (Cuellar 1984). Thus, viviparity may evolve more easily in taxa that are able to preclude storage of excess water in the extraembryonic compartment of the egg, while at the same time allowing retained embryos access to sufficient water for normal embryonic development.

Evolution of Viviparity in the scalaris *Species Group*

Viviparity has evolved at least twice within the *scalaris* species group (Sites et al. 1992). One case involves *Sceloporus aeneus* (oviparous) and its sister species *Sceloporus bicanthalis* (viviparous). These species differ in their reproductive modality, in the seasonal timing of reproduction, and in their distribution by elevation but are otherwise extremely similar (Guillette 1981, 1982; Guillette and Jones 1985). Our observations suggest that the transition from oviparity to viviparity may be relatively easy within the *scalaris* group. For *S. scalaris,* some of the constraints that limit the extension of egg retention in other species are apparently lacking. First, phenotypic plasticity in the duration of egg retention allows females to retain eggs for most of the developmental period. Second, such extended egg retention does not impede embryonic development. Finally, eggs that are retained beyond the time of normal oviposition take up relatively little water. Thus, at least one oviparous member of the *scalaris* group has reproductive attributes that would facilitate the evolution of viviparity.

Acknowledgments

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